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Influence of mulberry varieties on aminotransferase enzymes and economic parameters of the silkworm, *Bombyx mori* L

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Abstract
Success of Sericulture industry mainly depends on utilization of productive breeds and high nutritious mulberry leaves. Chemical composition of mulberry differs sharply among different mulberry varieties. The present study was conducted to know the influence of two mulberry varieties V_1 and S_{36} on aminotransferase enzymes as well as economic parameters in the silkworm breeds *viz*., CSR_2, CSR_4, NB_4D_2 and Diazo. The silkworm larvae reared with V_1 variety performed better in respect of larval weight, cocoon weight, shell weight, shell percentage, pupation rate, filament length, denier, renditta, reelability percentage and raw silk percentage as compared to S_{36}. Further, maximum activity of aminotransferase enzymes were registered in the silkworm larva reared on V_1 variety. However, the aspartate aminotransferase activity was more when compared to alanine aminotransferase in the silkworm batches reared with V_1 and S_{36} varieties.

Keywords: aminotransferase enzymes, *Bombyx mori* L., Economic parameters, Mulberry varieties, Silkworm

1. Introduction
India is the unique country which produces all four kinds of commercially available silk viz., tasar, eri, muga and mulberry. However, mulberry silk has greater demand because of its quality and mainly utilized for the production of fabric in textile industry. The silkworm being a monophagous insect it depends mulberry leaf as a source of food and synthesis 70 per cent of silk proteins. The cocoon yield is depends on number of factors which includes quality of silkworm egg, high yielding silkworm race/breed, maintenance of environmental condition, rearing technique and quality of mulberry leaf. Among these, mulberry leaf alone contributes (38.2%) for the success of cocoon crop (Miyashita, 1986) [16]. Nutritional composition of mulberry leaves varies with maturity and also due to environmental stress. It has been observed that the tender leaves are nutritive rich compared to medium and mature leaves (Rangaswami *et al.*, 1976) [19]. It is the protein and amino acid contents of mulberry leaf influence the shell weight (Mechii and Katagiri, 1991) [15]. Amount of leaf consumption and digestion has relationship with physical and chemical properties of leaves (Das and Vijayaraghavan, 1990 [8]; Ghosh *et al.*, 2000 [9]; Zannon *et al.*, 2012) [25]. The major nutrients like protein, amino acids, carbohydrate and lipid contents are required by the silk worm at larger proportion for their growth and development. These nutrients are converted into body elements with the involvement of various metabolic path ways.
The major transamination reactions are catalyzed by alanine aminotransferase (ALT, EC 2.6. 1.2) and aspartate aminotransferase (AST, EC 2.6. 1.2) enzymes which establish connecting link between carbohydrate and protein metabolism (Martin *et al*., 1981) [14] and their activities differ during varies physico-pathological condition (Nath *et. al*., 1997) [17]. There is a tissue grade variation of these enzymes in the silkworm. The higher levels of aminotransferase activities are reported in the silk gland as compared mid gut and fat body tissue (Horie and Nakamura., 1986) [11]. They also regulate the protein synthesis in different tissue by controlling supply of amino acids. It has been observed that mulberry leaf fortified with methionine enhance ALT and AST activities in the fat body of the silk worms larvae (Anil Kumar and Sunil Kumar, 2018) [3]. Similar trend is also noticed in the silkworm larvae supplemented mulberry leaf with soya bean flour, which not only enhances the enzyme activity but also economic parameters (Anil Kumar and Prashanth, 2018). Different species of mulberry may have compositional differences in nutrients and might lead to varying effects on *B. mori* growth and silk production (Mahmood *et al.*, 1987) [13]. The growth rate of *B. mori* larvae and subsequent silk production depends mainly on the nutrient contents of mulberry leaves. The nutritive value of mulberry leaves varies due to species and leaf maturity of the plant. The quality of mulberry leaf as a food for silkworm greatly affects the economy of sericulture industry (Das *et al*., 1983) [7]. It is well documented that, economic traits varies in different voltine groups of silk worm and also influenced by different mulberry varieties. However, not much information is available on effect of mulberry varieties on quantitative and qualitative traits as well as aminotransferase activity in silk worm breeds. Keeping this in view present investigation was initiated.

2. Materials and Methods
The silkworm breeds *viz*., CSR_2, CSR_4, NB_4D_2 and Diazo drawn from the Germ plasm bank Department of Studies in Sericulture Science, University of Mysore, Manasagangothri, Mysore the larvae of respective breeds were brushed and divided in two batches. Batch-I larvae were reared with V_1 mulberry variety and batch- II larvae were reared with S_{36} mulberry variety as per the standard rearing procedures of Basavaraja *et al*. (2002) [4]. A minimum of ten larvae (3 replications) from each batch

were used to estimate aminotransferase enzymes. In addition to this performance of the silk worm with respect to larval weight, cocoon weight, shell weight, shell ratio, filament length, denier, renditta, reelability percentage and raw silk percentage were also determined.

2.1 Estimation of alanine and aspartate aminotransferase enzymes.

The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity were estimated in fifth instar 1st day, 3rd day and prior to spinning stage (5th day) of silkworm breeds of both batch-I and Batch-II. The fat body tissue homogenate of 1% (w/v) was prepared by using distilled water and centrifuged at 3,000 rpm for 10 minutes; the crude extract supernatant was collected and used as a enzyme source. Both the enzymes were estimated by the method of Reitman and Frankel (1957) [20]. Aspartate aminotransferase was estimated by using 1ml of tissue extract was incubated with 0.5ml of glutamic oxalo acetate (substrate) at 37^0 C for 1 hour. To this reaction mixture, 0.5ml of 2, 4-D and 5ml of 0.4 N NaoH was added. The colour intensity was measured at 510 nm using spectrophotometer. For alanine aminotransferase, 0.5 ml glutamate pyruvate of was used as a substrate and standard curve was used for calculation. The enzyme activity was expressed in terms of units/g protein/h. The data obtained were analyzed by standard deviation (±) method and mean values were expressed. The parameters namely shell percentage, pupation rate; filament length, denier, renditta, reelability percentage and raw silk percentage were calculated by using following formulae.

$$\text{Shell ratio (\%)} = \frac{\text{shell weight (g)}}{\text{Coccon weight (g)}} \times 100$$

$$\text{Rate of pupation (\%)} = \frac{\text{Number of larvae pupated}}{\text{Number of larvae spun cocoons}} \times 100$$

$$\text{Filament Length (L)} = R \times 1.125$$

R = Number of revolutions recorded by epprouvette.

1.125 = Circumference of epprouvette in meter.

$$\text{Denier} = \frac{\text{Weight of the filament}}{\text{Length of the filament}} \times 9000$$

It denotes the thickness of the filament.

$$\text{Renditta} = \frac{\text{Weight of cocoons reeled}}{\text{Weight of raw silk obtained}}$$

Unit quantity of cocoon required to produce one unit of raw silk.

$$\text{Reelability} = \frac{\text{Number of cocoons taken for reeling}}{\text{No.of breakages+ No.of cocoons fed}} \times 100$$

$$\text{Raw silk percentage} = \frac{\text{Silk weight}}{\text{Green cocoon weight}} \times 100$$

3. Result and Discussion

3.1 Effect of mulberry varieties on alanine and aspartate aminotransferase activity

In insects, the fat body tissue is the site of intermediary metabolism and also serves as storage function of chief reserved substances like glycogen, protein and lipid, etc. In *B. mori*, various metabolites are synthesized in the fat body and it has contact with haemolymph, where in constant exchange of metabolites takes place between fat body with haemolymph and silk gland. The transaminases reaction is the important aspect of amino acid metabolism in which amino group is transferred from one amino acid to another amino acid with the aid of enzymes.

The aspartate (AST) and alanine (ALT) amino transferase which serves as connecting link between carbohydrate and protein metabolism Martin, *et al.* (1981) [14]. The aspartate aminotransferase activity (AST) level was maximum in the larvae of productive bivoltine breeds CSR_2 (410.48 units/g /protein/h) and CSR_4 (392.41 units/g protein/h) reared with V_1 variety. On the other hand minimum activity level was observed in Diazo (315.80 units/g protein/h) and NB_4D_2 (340.68 units/g protein/h) with S_{36} variety. Similarly, highest activity level of alanine aminotransferase was recorded in CSR_2 (210.39 units/g protein/h) and CSR_4 (184.31 units/g protein/h) with V_1 variety and it was lowest in Diazo (93.39 units/g protein/h) and NB_4D_2 (147.84 units/g protein/h) with S_{36} variety (Table. 1). Elevation in the activity of both AST and ALT enzymes in the fat body of silk worm breeds fed with V_1 variety over S_{36} variety indicates more active transport of amino acids their by helps in maintaining amino acid pool and also essential constituent for the synthesis of silk protein. These results are on line with the observations of Anil Kumar and Prashanth (2018) who have reported the increase in AST and ALT activities when silk worm larvae (CSR_2 and CSR_4) supplemented with soya bean floor at 7.5% concentration over remaining concentration as well as control batch. Similarly, Vinutha (2012) noticed that increase in total midgut protein content with V_1 variety when compared to S_{36} in different multivoltine and bivoltine silk worm breeds.

Irrespective of silk worm breeds the maximum activity levels of AST and ALT enzymes was noticed in fifth instar 5th day, followed by fifth instar 3rd day and fifth instar 1st day. It clearly shows that effective utilization of mulberry nutrients with the advancement of age. These results are on par with the earlier findings of Anil Kumar and Prashanth (2018) [2] who have noticed that AST and ALT activities increases in the silkworm larvae as the age proceeds towards conclusion of spinning. Similar trend was also noticed for protease activity in the silkworm larvae with advancement of age Anil Kumar (2009) [1]. Further, the silkworm breeds fed with V_1 variety, exhibit increase in AST and ALT activities indicating nutritional composition of V_1 variety is better than S_{36} variety (Table. 1).

3.2 Larval weight

Larval weight was greatly influenced by quality and quantum of mulberry leaves given to silk worm. It was found that highest larval weight was recorded in CSR_2 (3.968 g) and CSR_4 (3.822 g) with V_1 variety and it was lowest in Diazo (3.217 g) and NB_4D_2 (3.518 g) with S_{36} is due to better nutritional value of V_1 might influence of this trait (Table. 2). These results are also supported by the observations of Tayade and Jwala (1984) [23], Boraiah *et al.* (1987) [6], Srivastava [21] and Elangovan (2007) and Vinutha (2012) [24] who have reported that larva reared with different mulberry varieties exhibit variation in the larval weight

3.3 Cocoon weight

Silk worm breeds reared on two mulberry varieties exhibited marked impact on cocoon weight. The CSR_2 and CSR_4 express higher cocoon weight of 2.318 g and 1.921 g with V_1 variety, respectively (Table. 2). The increase in cocoon weight in both breeds might be due to higher

nutrient content of V_1 variety when compared to S_{36}. The results are in conformity with those of Bheemanna *et al.* (1997) [5] who have reported that cocoon weight was highest when larvae fed with S-41, Mysore local and S-36 varieties, respectively. Further, observations made by Haque *et al.* (1990) [10] indicated that cocoon weight of selected races, Nistari, Nan-nung, Nistid white, BSRI-801 and O-5 showed maximum results with BM-1 variety followed by BM-3, BSRM-4 and BM-2 varieties. Similar trends were also noticed by Vinutha (2012) [24] in some other multivoltine and bivoltine breeds.

3.4 Shell weight
Cocoon shell formed by the silk worm breeds with two mulberry varieties registered considerable variations in respect of shell weight. It was noticed that maximum shell weight of 0.500 g in CSR_2 and 0.398 g in CSR_4 with V_1 variety. In contrast it was lowest in Diazo (0.159 g) when the larvae reared with S_{36} (Table. 2). The gain in the shell weight may be due to higher nutritive value of V_1 over S_{36} variety. These results are in agreement with those of Bheemanna *et al.* (1997) [5] who have opined that this trait was highest when the larvae fed with S-4, Mysore local and S-36 variety respectively. Further, observations made by Haque *et al.* (1990) [10] indicated that the shell weight of selected races Nistari, Nan-nung, Nistid white, BSRI-801 and O-5 showed higher shell weight with BM-1 variety followed by BM-3, BSRM-4 and BM-2 varieties. Similar observation was also registered by Vinutha (2012) [24] in some other multivoltine and bivoltine breeds reared with V_1 and M_5 varieties.

3.5 Shell percentage
It depicts the actual silk recovery from the cocoons and it varies with the mulberry varieties and silk worm breeds. The highest shell percentage was recorded in CSR_2 (21.94 g) and CSR_4 (20.72 g) with V_1 variety. On the other hand it was lowest in Diazo (12.22 g) and NB_4D_2 (18.77 g) with S_{36} (Table. 2). These results are on line with the earlier observations of Bheemanna *et al.* (1997) [5] who have revealed that this trait was maximum when the larvae fed with S-41, Mysore local and S-36 variety, respectively. Further, observations made by Haque *et al.* (1990), indicated that the shell percentage of selected races Nistari, Nan-nung, Nistid white, BSRI-801 and O-5 showed highest results with BM-1 variety followed by BM-3, BSRM-4 and BM-2 varieties. This type of result was also observed by Vinutha (2012) in some other silk breeds fed with V_1 over M_5 variety.

3.6 Pupation rate
This is an important yield attributed parameter and it denotes the viability which is expressed in terms of percentage. The pupation rate was maximum in CSR_2 and CSR_4 (91%) with V_1 variety. The same was minimum in Diazo (80%) and NB_4D_2 (85%) when the larvae reared with S_{36} variety (Table. 2). These results are in agreement with the findings of Sudharshana Reddy (1999) [22] who has reported that the pupation percentage was highest in the silk worm hybrid PM × NB_{18} fed with S-54 and it was lowest with S-41 leaf. Similar observation was also obtained by Vinutha (2012) [24] for the said trait in the silk worm larvae fed with V_1 variety performed well when compared to M_5.

3.7 Filament length
Filament length is one of the major contributory quantitative traits in silk worm. The longest filament length was recorded in CSR_2 (1093.0 m) and CSR_4 (983.2 m) with V_1 variety. As against to this it was shortest in NB_4D_2 (823.4 m) and Diazo (450.2 m) with S_{36} (Table. 2). These results are in conformity in the findings of Bheemanna *et al.* (1997) [5] who have inferred that the filament length was longest when the larvae fed with S-54, S-36 and S-41, respectively. Further, observations made by Haque *et al.* (1990) [10], the filament length of selected silkworm races viz., Nistari, Nan-nung, Nistid white, BSRI-801 and O-5 showed better results in batches fed with BM-1 followed by BM-3, BSRM-4 and BM-2 varieties. Similar trend was also noticed by Vinutha (2012) [24] in some other silk breeds fed with V_1 variety over M_5 variety.

3.8 Denier
Denier is the important trait identifying silk quality which denotes thickness of the filament. Thinner denier was registered in Diazo (2.11) and CSR_2 (2.16) with V_1 variety. On the other hand, CSR_4 (2.51) and CSR_2 (2.30) registered thicker denier with S_{36} (Table. 2). These corroborates the earlier findings of Narayanan *et al.* (1966) [18] who have opined that when silk worm race, Mysore princes was fed with Kanava-2 leaves recorded coarser denier over local leaf in two out of three rearing. Similarly, Vinutha (2012) [24] noticed that the silk worm breeds reared with V_1 variety performed better for this trait as against M_5.

3.9 Renditta
This trait mainly depends on cocoon weight and shell content. Higher the shell content and pupal weight lower will be the renditta. The lowest renditta was noticed in CSR_2 (6.46) and CSR_4 (6.16) with V_1 variety (Table. 2). This shows that the productive bivoltine breeds utilizes mulberry nutrients effectively as compared to other multivoltine breed. Irrespective of the breeds, encouraging results was obtained in the batches of silk worm larvae fed with V_1 variety for the said trait. It clearly depicts that the superiority of V_1 variety pertaining to nutrients when compared to S_{36}. These results are also supported by the observation of Vinutha (2012) [24] who reported that silk worms fed with V_1 variety excelled over S_{36} variety for this trait.

3.10 Reelability percentage
Reelability is directly correlated with non-broken filament length. Longer the non-broken filament length higher will be the Reelability. The maximum Reelability percentage was registered in productive breeds CSR_2 (84.29%) and CSR_4 (80.95%) with V_1 variety and it was lowest in Diazo (59.01%) with S_{36} (Table. 2). These results are supported by the observations of Mallikarjunappa *et al.* (2000) [12] who have observed that, the mulberry variety S-30 performed well with respect to reelability percentage in case of NB_7 × NB_{18} when compared to S-36, Viswa and M_5 varieties. Similar trend was also observed by Vinutha (2012) [24] in the silk worm breeds reared with V_1 variety performed better for this trait as compared to S_{36}.

3.11 Raw silk percentage
The raw silk percentage is the prime factor which depicts the productivity of the race / breed. The raw silk percentage

of 18.54 and 80.95% was expressed in CSR_2 with V_1 and S_{36} varieties, respectively. It was lowest in Diazo (10.31%) with S36 variety (Table. 2). These results are in agreement with the earlier observations of Mallikarjunappa *et al.* (2000) [12] who have reported that, the mulberry variety S-30 performed well with respect to all economic characters including raw silk percentage in $NB_7 \times NB_{18}$ as against S-36, Viswa and M_5 varieties. This type of observations was also made by Vinutha (2012) [24] in the silk worm breeds reared with V_1 variety excelled over S_{36} for this trait.

Table 1: Effect of feeding mulberry varieties on rearing and reeling parameters of silk worm breeds

Mulberry variety	Breed	Larval weight (g)	Cocoon Weight (g)	Shell Weight (g)	Shell Ratio (%)	Pupation Rate (%)	Filament Length (m)	Denier	Renditta	Reelability (%)	Raw Silk Percentage (%)
V1	CSR2	3.968 ± 0.01	2.318 ± 0.21	0.500 ± 0.08	21.94 ± 1.48	92	1093 ± 34.74	2.16 ± 0.20	6.46 ± 0.02	84.29 ± 10.19	18.84 ± 1.35
	CSR4	3.822 ± 0.01	1.921 ± 0.10	0.398 ± 0.04	20.72 ± 5.31	92	983.2 ± 5.80	2.40 ± 0.12	6.61 ± 0.08	80.95 ± 5.32	17.65 ± 1.31
	NB4D2	3.640 ± 0.009	1.582 ± 0.16	0.297 ± 0.04	18.80 ± 4.60	88	864.0 ± 11.93	2.27 ± 0.14	7.06 ± 0.062	79.00 ± 9.50	16.26 ± 1.42
	Daizo	3.451 ± 0.014	1.470 ± 0.12	0.197 ± 0.004	13.40 ± 0.96	82	466.0 ± 11.20	2.11 ± 0.17	10.32 ± 1.13	61.51 ± 6.54	10.78 ± 1.10
S36	CSR2	3.902 ± 0.012	2.301 ± 0.21	0.482 ± 0.06	21.57 ± 4.87	90	1042.8 ± 21.42	2.30 ± 0.11	6.57 ± 0.006	80.95 ± 5.32	17.96 ± 2.11
	CSR4	3.786 ± 0.01	1.646 ± 0.12	0.340 ± 0.05	20.71 ± 4.10	89	924.8 ± 24.75	2.51 ± 0.33	6.93 ± 0.05	79.29 ± 9.76	17.08 ± 1.57
	NB4D2	3.518 ± 0.009	1.508 ± 0.15	0.274 ± 0.04	18.77 ± 2.04	85	823.4 ± 22.42	2.35 ± 0.13	7.29 ± 0.018	78.59 ± 6.52	16.24 ± 1.93
	Daizo	3.217 ± 0.007	1.301 ± 0.08	0.159 ± 0.01	12.22 ± 0.49	80	450.2 ± 5.54	2.22 ± 0.34	10.84 ± 1.18	59.01 ± 8.23	10.31 ± 0.10

Table 2: Effect of feeding mulberry varieties on aminotransferase enzymes in the silk worm breeds

Mulberry variety	Breed	Alanine aminotransferase activity		
		Days		
		1st Day	3rd Day	5th Day
V_1	CSR_2	190.18 ± 0.48	316.48 ± 0.20	410.48 ± 0.30
	CSR_4	150.22 ± 0.43	299.70 ± 0.18	392.41 ± 0.20
	NB_4D_2	110.65 ± 0.26	262.60 ± 0.15	358.71 ± 0.15
	Daizo	98.30 ± 0.20	232.33 ± 0.34	326.61 ± 0.14
S_{36}	CSR_2	169.75 ± 0.45	304.65 ± 0.40	340.69 ± 0.20
	CSR_4	130.49 ± 0.39	280.68 ± 0.25	337.66 ± 0.20
	NB_4D_2	109.43 ± 0.20	250.40 ± 0.31	340.68 ± 0.15
	Daizo	92.65 ± 0.24	195.37 ± 0.35	365.80 ± 0.14
Aspartate aminotransferase activity				
V_1	CSR_2	134.74 ± 0.26	169.69 ± 0.388	210.39 ± 0.223
	CSR_4	120.02 ± 0.215	148.50 ± 0.291	184.31 ± 0.376
	NB_4D_2	96.19 ± 0.106	120.47 ± 0.226	162.08 ± 0.487
	Daizo	82.02 ± 0.071	95.30 ± 0.541	120.79 ± 0.413
S_{36}	CSR_2	110.33 ± 0.189	165.65 ± 0.257	195.50 ± 0.412
	CSR_4	99.99 ± 0.134	132.54 ± 0.290	178.46 ± 0.379
	NB_4D_2	88.80 ± 0.525	103.35 ± 0.225	147.84 ± 0.391
	Daizo	78.31 ± 0.204	90.52 ± 0.323	93.39 ± 0.274

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